General Variant Classification/ Assertion criteria

General Information

Variant identification and interpretation is a critical step in making genetic diagnosis and personalized medicine a reality. The in-house method of variant classification at K & H Personalized Medicine Clinic® complies with the American College of Medical Genetics (ACMG) standards. We devised standard internal guidelines, to assess the robustness of publicly available information, gene-disease relationship, the clinical impact of nucleotide variations, the availability of treatments, and preventive measures. Internal criteria are designed to refine ACMG/AMP guidelines based on latest data available for assessing the strength of the variant and most recent information specific to genes & gene-phenotype association.

K & H Personalized Medicine Clinic® variant interpretation features a combination of open source tools with automated in-house algorithms. A wide range of information from public resources and in-house databases is retrieved through machine learning approaches, and in-depth evaluation is done by a team of qualified professionals.

In the assessment of the variant classification, K & H Personalized Medicine Clinic® considers information and evidence that includes, but is not limited to, the following 5 major parameters. The functional impact of the gene in causing the disease phenotype, functional impact of variation in the gene product based on *in silico*, *in vitro* and *in vivo* studies, variant-disease association, prevalence and significance. All information derived from peer-reviewed published literature, and in-house database testing, are considered when weighing a variant in favor of pathogenicity.

Variant Classification

K & H Personalized Medicine Clinic® classifies the variants into 6 categories as per ACMG guidelines and based on the above criteria. Variants are categorized under each of these categories based on the consistency of the evidence. It should be noted that documentation can make any early evidence obsolete.

1. ***Pathogenic variants***:

A variant which is more likely to cause a disease is defined as pathogenic variant. All the variants which can cause loss of protein function or predictable significant damage to the gene product or can alter protein/protein interactions fall under this category.
SNPs, Indels and CNVs resulting in are primary variant types considered. The modifier effects of the primary variants are then identified. Further those affecting the gene product function i.e. variants that could lead to truncation of the gene product such as nonsense variant, frameshift alterations, stop gain variants and variants that can affect the canonical splice acceptor and splice donor sites, are classified as pathogenic. Such variants which have significant genotype-phenotype relationship and are highly prevalent in disease phenotype when compared to unaffected individuals or control group are also considered to be pathogenic. In-silico tools such as SIFT score, Polyphen-2 score and data from MutationTaster are utilized to predict the pathogenicity, strength and impact of variation on protein function of the variant.

2. ***Likely pathogenic variants:***

Variants which are located in functional domains of the protein with high or moderate variant impact and are frequently prevalent in observed phenotype when compared to healthy population, without demonstrated significance from case-control studies yet are categorized as likely-pathogenic. The frequency of prevalence is obtained from open source databases control data, from peer reviewed publications and internal data review.

3. ***Risk factor variants:***

Probably damaging Missense variants, intronic variants, UTRs with moderate variant impact, and show significant genotype-phenotype association are considered to be risk factor variants. These variants are known to increase an individual's susceptibility or predisposition to a certain disease phenotype.

4. ***Benign:***

Variants of low or moderate variant impact (from *in silico* studies) which are known to have a significant genotype-phenotype relationship as evident from *in vitro* and or *in vivo* studies and a not known to cause the disease are considered as variants of benign clinical significance. In-frame deletions or insertions in repetitive regions without a known function are also considered as variants of benign significance.

5. ***Likely Benign:***

Any variants with conflicting interpretations of association with the disease phenotype are weighed carefully for the functional effect of the variant and number of affected individuals when compared to healthy population. If the variant is predicted to have low impact, have low variant impact, is tolerated mutation in *insilico* observations with conflicting evidences or have inadequate clinical information, they are classified as variants of likely benign clinical significance. Low allele frequency variants consistent with particular ethnic groups are also considered as likely benign.

6. ***Uncertain significance:***

Variants that have insufficient evidence or multiple studies with opposing results, to indicate it is likely benign or likely pathogenic for a given phenotype are classified as variant of unknown or uncertain significance. In frame deletion alterations, Synonymous (intron variants other than splice site variants), missense variations which have low variant impact and do not have functional genotype-phenotype association are classified under variants of uncertain significance. Variants which have known evidence of gene-phenotype relationship and no sufficient reported evidence on variant- phenotype association are also categorized as Variants of Uncertain Significance.

Variants with sufficient reported evidence of non-association with the phenotype from both internal and external sources, any novel silent variants which do not show genotype –phenotype association are also classified as Variants of Uncertain Significance.

For rare disorders, proportionally lower allele frequencies are accepted as stand- alone criteria relative to the disease incidence. Open source population databases like 1000 Genomes, NHLBI Exome Sequencing Project (ESP) Exome Variant Server, Exome Aggregation Consortium (ExAC) are screened for frequency of the variant in control population. Additional databases, computational tools and in-house algorithms are tested and considered as new sources of information become available.

Extensive interactions between the variant classification team, molecular biologists, bioinformaticians and genetic counselors ensure continual progress in variant classification quality that facilitates the accuracy of classification results. All variant classifications are re-assessed at defined intervals for relevant updates that may influence the interpretation of the final report. Final reports are reviewed and approved according to clinical indications from the research director.

References:

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